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ABSTRACT. Tissue samples obtained from the lateral wall of the maxillary sinuses of five patients were examined by light microscopical, histochemical, and ultrastructural techniques. Submucosal glands were tubulo-alveolar mixed glands. The acini consisted of either all serous or all mucous cells, or of a mixture of both cell types. Serous granules were stained by toluidine blue or by hematoxylin and eosin, but showed little or no reaction with the PAS-reagent or with Alcian blue. Mucous granules were pale in toluidine blue or H and E preparations, and consisted primarily of acid mucosubstances, as demonstrated by their staining reaction with PAS and Alcian blue. At the electron microscope level, the serous granules were either homogeneously dense, or showed a substructure consisting of at least two layers of distinctly different electron-opacity. Typical mucous droplets consisted of a fibillar network dispersed in a translucent matrix. A second secretory product was present in the mucous cells in the form of elongated, membrane-bounded structures containing numerous, parallel filaments, which measured about 55A in diameter. The mucous droplets and the filamentous bodies appear to arise from the opposite faces of the Golgi complex in the mucous cells. The filamentous bodies showed a pronounced tendency to fuse with the mucous droplets. All acini were surrounded by a well-defined myoepithelial layer and contained intercellular nerve terminals.

Many different exocrine glands are present in the submucosa of the nasal passages. Of these, only Bowman's glands (Frisch, '67; Seifert, '71b; Breipohl, '72), anterior nasal glands (Kerjaschki, '74), and vomeronasal glands (Seifert, '71a; Tandler and Bojsen-Møller, '74) have been examined by electron microscopy. In the maxillary sinuses, the lateral nasal (Steno's) gland (Moe and Bojsen-Møller, '71; Vidić and Taylor, '72) and the maxillary gland of the rat (Vidić and Greditzer, '71; Vidić, '73) have received some attention, but the dispersed mixed glands underlying the human sinus epithelium have been largely overlooked.

According to Sappey (1889) the submucosal glands are distributed throughout all of the walls of the sinus cavity. They are described as pleomorphic with respect to their size and acinar configuration. Although the submucosal glands are abundant and may make a significant contribution to nasal secretions, they have been virtually ignored since Sappey's time. Most modern textbooks of otorhinolaryngology acknowledge that there are such glands in the sinus submucosa, but give no histological description of these organs despite their possible role in nasal function.

The present article describes the histology, histochemistry and ultrastructure of these submucosal glands in the human maxillary sinus.

MATERIALS AND METHODS

Tissue specimens were obtained by surgery from five adult patients at the District of Columbia General Hospital under the auspices of the Department of Otolaryngology.** The ablation of soft tissues from the maxillary sinus, was undertaken in each

instance, because inverted papillomas originating from the medial wall of the sinus interferred with the ostium or with the communication of the sinus with the middle nasal meatus. Following general anesthesia and a local application of neosynephrine, the sinuses were perforated through the anterior wall from the oral vestibule, and several samples of grossly normal lining were removed from the lateral wall of the sinus. Samples were fixed for two hours at 4°C in a 3 to 6% solution of glutaraldehyde-paraformaldehyde (Ito and Karnovsky, '68) buffered to pH 7.2 with 0.1 M sodium cacodylate. The samples were then washed in buffer for 24 hours, postfixed for two hours at 4°C in a 2% aqueous solution of osmium tetroxide, stained in block for one hour with 1% uranyl acetate buffered with maleic acid to pH 6.0, dehydrated, and embedded in Epon. Thin sections were stained with heavy metal salts (Watson, '58; Venable and Coggeshall, '65) and observed in an AEI-EM 801 electron microscope.

For light microscopy, epoxy sections 0.5-1.0 mμ thick were stained with toluidine blue (Pearse, '68). A portion of each tissue specimen was fixed in formalin, embedded in paraffin, and sectioned at 5 mμ. These sections were stained with hematoxylin and eosin, periodic acid-Schiff and hematoxylin, or Alcian blue and nuclear fast red, and studied with a Leitz microscope.

OBSERVATIONS

Light microscopy. The submucosa of the human maxillary sinus contained dispersed exocrine glands of the tubuloalveolar type (figs. 1, 2). Both serous and mucous cells were present in the glandular acini. In some acini, these cell types were intermingled, so that

both serous and mucous cells bordered on the same lumen. Other acini consisted entirely of either mucous or serous cells. Mucous acini were sometimes capped by a serous demilune. Flattened myoepithelial cells were distinguishable at the acinar perimeter.

Mucous cells were characterized by a pale cytoplasm and a flattened, basally located nucleus. These cells contained abundant mucosubstances (pH range from 0.5-4.5), as demonstrated by their strong staining by the periodic acid-Schiff and Alcian blue procedures. The serous cells had a rather basophilic cytoplasm and a round nucleus usually situated in the basal third of the cell. These cells were only weakly PAS-positive, and showed no reactivity towards Alcian blue. Serous demilune cells were flatter than their acinar counterpart, but showed the same staining properties.

Electron microscopy. In serous acini or in mucous acini lacking serous demilunes, the secretory cells had the same general shape--they were columnar to cuboidal and somewhat narrower at their apex than at their base. Both serous and mucous cells bore microvilli on their apical surface, with those on the serous cells showing an obvious filamentous core. The acinar lumina ran an irregular course, so that the same lumen often was intersected several times in the plane of a single section. Adjacent cells demonstrated a considerable degree of lateral interdigitation. Basal folds were often present on the basal surface of the secretory cells, and these cells frequently rested on a myoepithelial cell or on several myoepithelial processes. Serous demilune cells had the same morphological features as the serous acinar cells, but were considerably flatter.

The serous cells possessed all of the morphological hallmarks

usually associated with serous-type cells, viz., an extensive granular endoplasmic reticulum, prominent Golgi complexes, abundant mitochondria, and numerous membrane-bounded secretory granules. The serous granules in the submucosal gland cells assumed a variety of appearances based on the density and distribution of their content (figs. 3, 4, 5, 6, 7). The most typical granules were moderately dense and homogeneous in appearance. In other cells, the granules often consisted of a light central region surrounded by a halo of substantially denser material. Certain other granules had the same arrangement of light and dark zones, but the difference in density between the two components was less pronounced. Furthermore, a distinct dark band served to separate the two zones from each other. In still other cells, the light and dark areas of the granule matrix were reversed so that the center of the granules was the darkest portion, with the periphery being lighter. Whatever their internal organization, the serous granules appeared to be liberated into the acinar lumen by a typical merocrine process (fig. 8).

The most outstanding feature of the mucous cells was the abundance of mucous droplets that almost completely filled the supranuclear cytoplasm. Only a few scattered organelles were evident in the interstices between the closely-packed droplets. Typical mucous droplets were large (about 2 mμ in diameter), membrane-bounded, and showed a marked propensity for lateral fusion with neighboring droplets. They consisted of finely fibrillar material disposed in an irregularly reticulate pattern within a low-density matrix. The packing of the fibrillar meshwork appeared to be related to the degree of maturity of the droplets, being looser in

the less mature droplets. In some mucous cells, the droplets contained a fairly dense spherule, which often was eccentrically placed (figs. 9, 10, 11).

A second type of secretory granule, which tended to be elongated in shape rather than round, was present within virtually all mucous cells. These membrane-delimited bodies consisted of numerous parallel filaments (fig. 12), each measuring approximately 55Å in diameter, suspended in a low-density matrix. Viewed in transverse-section, the filaments appeared as dots (fig. 13). Small dense structures resembling lipid droplets were frequently scattered among the fibrils. Occasionally, these structures attained substantial size, and their resemblance to lipid was correspondingly enhanced (fig. 14). The filamentous bodies also demonstrated a proclivity for fusion with mucous-droplets (fig. 15); obvious fusion figures were often observed.

Both the mucous droplets and the filamentous bodies appeared to arise in association with the selfsame Golgi complex. On one face of this organelle, that associated with mucous droplets, the outermost cisternae appeared to be dilated, and contained finely filamentous material matching that in the droplets. On the opposite face of the Golgi complex, that facing the filamentous bodies, the cisternae contained fibrillar material resembling that in the bodies (fig. 16).

Nerve terminals (fig. 17) were often observed in an intercellular position. These frequently contained mixed populations of vesicles--large dense-cored vesicles and small apparently empty vesicles. Myoepithelial cells were typical in appearance, containing numerous myofilaments (fig. 18) and showing many pinocytotic vesicles at their surfaces.

DISCUSSION

Based on their staining reactions and on their ultrastructure, the acini of the submucosal glands of the human maxillary sinuses are seen to consist of either serous or mucous cells. These cells bear a strong resemblance to their counterparts in a variety of human salivary glands. Rather than being homogeneous structures, the serous secretory granules in the submucosal glands display a striking variability in their internal organization. While this variability in granule appearance within individual cells may be due in part to a maturational sequence, clear differences in granules are apparent in neighboring cells. Thus, in one cell the granules may have a dense rim with a light central zone, while in an adjacent cell the granules may have a light rim with a dense central zone, not to mention several other possible configurations. These differences in appearance suggest that even contiguous serous cells in submucosal glands may be producing somewhat different secretory products. A similar situation occurs in the serous cells of the human submaxillary gland (Tandler and Erlandson, '72). In the hamster submandibular gland, the appearance of the seromucous cells seems to be sex-linked (Dorey and Bhoola, '72). In the female hamster, these granules show a moderately-dense cortical region surrounding a pale inner area, while in the male the situation is reversed.

It has become apparent in the last few years that secretory granules with obvious substructure occur with far greater frequency than do granules with a uniform matrix; some of the manifold

patterns assumed by secretory granules have been catalogued by Tandler and MacCallum ('72). The significance of matrix zonation in serous-type granules is not clearly understood. Digestion of thin sections with pronase has demonstrated that the complex organization of granules in lingual granules of the salamander is due to ordered disposition of the protein and mucosubstance components of the granule matrix (Zylberberg, '73). Similarly, histochemical tests carried out on thin sections have shown that acid mucosubstances are restricted to the electron-lucent rim of serous granules in the parotid and sublingual glands of the Mongolian gerbil (Ichikawa and Ichikawa, '74). The question has been posed whether or not this sequestration of stainable components in secretory granules is a reflection of the presence of discrete aggregates of different enzymes within individual granules (Tandler and Erlandson, '72). This question can be answered only after granules with a well-defined substructure are successfully isolated and analyzed.

The finely-fibrillar reticulate appearance of the content of mucous droplets, in the maxillary sinus submucosal glands, is similar to that of droplets in mucous and seromucous cells in different types of salivary glands; the stained material in the secretion of the latter organs has been suggested to be glycoproteins (Gallagher, Marsden and Robards, '69). In the submucosal glands, the mucous droplets show little of the range in density found in human labial salivary glands (Tandler et al., '69) or of the staining variations in submucosal glands in the human tracheo-bronchial tree (Lamb and Reid, '69). Occasional clusters of these droplets, however, may

contain a dense spherule, an indication that there may be some differences in their composition. The submucosal mucous droplets show a strong tendency to fuse, producing relatively large, irregular, membrane-bounded aggregates of mucus. This process has been implicated in the extrusion of mucous secretions in the rat sublingual gland (Kim, Nasjleti and Han, '72).

In addition to mucous droplets, the mucous cells consistently contain at least a few, and sometimes many, filamentous bodies. These are identical in morphology to certain cytoplasmic structures found in mucous cells of human sublingual and submandibular glands (Tandler, unpublished observations) and of human palatine salivary glands (Shimono et al., '73). They also resemble the duplex inclusions described in human labial salivary glands (Tandler et al., '69) and the Biondi-like inclusions found in the human iris (Ringvold, '74), although the lipid component usually is less prominent in the submucosal gland filamentous bodies. Similar structures are present in mucous cells of the human endocervix, where it has been claimed (without any direct evidence) that they arise by transformation of ordinary mucous droplets by an irreversible process (Philipp, '72). From our observations, it seems obvious that the filamentous bodies represent a second type of secretion produced by the mucous cells of the submucosal glands of the maxillary sinus. The Golgi complex in these cells appears to simultaneously produce two distinct secretory products from its two faces; on one side, mucous droplets; on the other, filamentous bodies. The production of two discrete secretory products from the same Golgi complex is not without precedent; a similar finding has been documented in

polymorphonuclear leukocytes, where both specific and azurophil granules arise in association with the Golgi complex, although in this case, the two secretory products are produced at different stages in development of the cells (Bainton and Farquhar, '66).

According to Philipp ('72), the filamentous bodies remain in endocervical cells when mucous is extruded. The ostensible absence of filamentous material in the gland lumen may be explained by our finding of fusion between filamentous bodies and mucous droplets, wherein the filaments appear to depolymerize. As a consequence, the biochemical components of the filaments are present within modified mucous droplets, albeit in a no longer recognizable form, and are available for release. The mechanism of filament depolymerization is unknown. It could be based on exposure to a change in pH of the secretory droplet as a result of fusion, or on exposure to enzymatic activity within the mucous.

FOOTNOTE

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FOOTNOTE

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LITERATURE CITED

Bainton, D. F., and M. G. Farquhar 1966 Origin of granules in polymorphonuclear leukocytes. Two types derived from opposite faces of the Golgi complex in developing granulocytes. J. Cell Biol., 28: 277-301.

Breipohl, W. 1972 Licht- und elektronenmikroskopische Befunde zur Struktur der Bowmanschen Drüsen im Riechepithel der weissen Maus. Z. Zellforsch., 131: 329-346.

Dorey, G., and K. D. Bhoola 1972 I. Ultrastructure of acinar cell granules in mammalian submaxillary glands. Z. Zellforsch., 126: 320-334.

Frisch, D. 1967 Ultrastructure of the mouse olfactory mucosa. Am. J. Anat., 121: 87-120.

Gallagher, J. T., J. C. Marsden and A. W. Robards 1969 Electron microscopic investigations of submaxillary salivary gland glycoproteins. Arch. Oral. Biol., 14: 731-734.

Ichikawa, A., and M. Ichikawa 1974 Fine structural analyses of the serous secretory granules in the salivary glands of the Mongolian gerbil. Proc. 8th International Congress on Electron Microscopy, Canberra. Vol. II, pp. 440-441.

Ito, S., and M. J. Karnovsky 1968 Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. J. Cell Biol., 39: 168A-169A.

Kerjaschki, D. 1974 The anterior medial gland in the mouse nasal septum: an uncommon type of epithelium with abundant innervation. J. Ultrastruct. Res., 46: 466-482.

Kim, S. K., C. E. Nasjleti and S. S. Han 1972 The secretion processes in mucous and serous secretory cells of the rat sublingual gland. J. Ultrastruct. Res., 38: 371-389.

Lamb, D., and L. Reid 1969 Histochemical types of acidic glycoprotein produced by mucous cells of the tracheobronchial glands in man. J. Path., 98: 213-229.

Moe, H., and F. Bojsen-Møller 1971 The fine structure of the lateral nasal gland (Steno's gland) of the rat. J. Ultrastruct. Res., 36: 127-148.

Pearse, A. G. 1968 Histochemistry. Vol. I. Little, Brown and Co., Boston, p. 665.

Philipp, E. 1972 Uber den granulofilamentaren Umbau von Sekretgranula im schleimbildenen Epithel der Endocervix der Frau. Z. Zellforsch., 134: 555-563.

Ringvold, A. 1974 Biondi-like cell inclusions in the human iris. Acta Ophthalmol., 52: 541-550.

Sappey, P. C. 1889 Traite d'Anatomie Descriptive, 4th edition, Vol. 3. Lecrosnier et Babe, Paris, pp. 653-656.

Seifert, K. 1971a Licht- und elektronenmikroskopische Untersuchungen am Jacobsonschen Organ (Organon vomero nasale) der Katze. Arch. Klin. Exp. Ohr. Nas. Kehlk. Heilk., 200: 223-251.

------ 1971b Licht- und elektrononmikroskopische Untersuchungen der Bowman-Drüsen in der Riechschleimhaut makrosmatischer Sauger. Arch. Klin. Exp. Ohr. Nas. Kehlk. Heilk., 200: 252-274.

Shimono, M., E. Hasegawa, H. Yamane, H. Kawahara, S. Eda, T. Yamamura, G. Yamane, T. Kato and T. Okuyama 1971 Ultrastructure of the human salivary glands. 1st report: The acinar cells. Shikwa Gakuho, 71: 369-380.

Tandler, B., and F. Bojsen-Møller 1974 Ultrastructure of the submucosal glands of the rat nasal septum. Anat. Rec. (abstract), 178: 474.

Tandler, B., C. R. Denning, I. D. Mandel and A. H. Kutscher 1969 Ultrastructure of human labial salivary glands. I. Acinar secretory cells. J. Morph., 127: 383-408.

Tandler, B., and R. A. Erlandson 1972 Ultrastructure of the human submaxillary gland. IV. Serous granules. Am. J. Anat., 135: 419-434.

Tandler, B., and D. K. MacCallum 1972 Ultrastructure and histochemistry of the submandibular gland of the European hedghog, Erinaceous europaeus L. I. Acinar secretory cells. J. Ultrastruct. Res., 39: 186-204.

Venable, J. H., and R. Coggeshall 1965 A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25: 407-408.

Vidić, B., and H. G. Greditzer 1971 The histochemical and microscopical differentiation of the respiratory glands around the maxillary sinus of the rat. Am. J. Anat., 132: 491-514.

Vidić, B., and J. J. Taylor 1972 The structure of the acinar cell and its relationship to the nerve terminals in the lateral nasal gland of the rat. Arch. Histol. Japon., 34: 449-461.

Vidić, B. 1973 Structure and cytochemistry of the acinar cell in the rat maxillary gland. Am. J. Anat., 137: 103-118.

Watson, M. L. 1958 Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol., 4: 475-478.

Zylberberg, L. 1973 Donnees histologiques sur les glandes linguales de quelques Salamandrides (Batraciens, Urodeles). Arch. Biol., 84: 341-366.

PLATE 1

EXPLANATION OF FIGURES

1 This photomicrograph of the submucosal layer from the maxillary sinus demonstrates the general arrangement of serous (S) and mucous (M) secretory cells in several acini. The dark cells at the acinar periphery are myoepithelial cells (arrows). Toluidine blue. X 675.

2 Several acini composed entirely of either serous (S) or of mucous (M) secretory cells are indicated in this section. In addition, three acini (arrows) converge toward a common duct. Periodic acid-Schiff and hematoxylin. X 675.

3 An electron micrograph illustrating the diverse appearance of secretory granules in several adjoined cells. Note the relatively uniform ultrastructure of secretory granules within any individual acinar cell. Acinar lumina (L) are also shown. X 8,000.

PLATE 2

EXPLANATION OF FIGURES

4, 5, 6, 7 These micrographs illustrate the various appearances assumed by secretory granules (G) in different serous cells. Some granules (fig. 4) contain a homogeneous electron-opaque substance. The other granules show two substances of distinctly different structure and opacity. Fig. 4, X 18,400; Fig. 5, X 22,500; Fig. 6, X 17,000; Fig. 7, X 48,000.

8 A serous granule (G) in the apical cytoplasm is closely apposed to the plasmalemma (arrow), suggesting initiation of membrane fusion preceding the extrusion of secretory material into the acinar lumen (L). X 80,000.

PLATE 3

EXPLANATION OF FIGURES

9 The Golgi region of a mucous cell. Many of the droplets surrounding the Golgi apparatus (arrowhead) are in various stages of coalescence (arrows). X 20,000.

10 The internal structure of a mucous droplet. The granular membrane is indicated by arrow. X 125,000.

11 Inclusions of electron-opaque material (arrowheads) are found in some mucous granules. X 43,000.

PLATE 4

EXPLANATION OF FIGURES

12 In this mucous cell, two types of secretory products are demonstrated. Typical mucous droplets (G) contain electron-opaque fibrils arranged in a network-like pattern. The other type of granule (arrows) contains parallel filaments. Small droplets, presumably lipid in character, are sometimes present in the filamentous bodies. X 18,000.

13 Filaments are demonstrated in perpendicular (P) and oblique (O) sections in the same secretory droplet. Possible initial fusion of this granule with an ordinary mucous droplet is indicated by the arrow. X 80,000.

14 Several filamentous bodies are present in this mucous cell. A large droplet (D) is bounded by the same membrane (arrow) that delimits one of the filamentous bodies. This presence within these bodies of lipid droplets of varying sizes is a common occurrence in the submucosal gland mucous cells. X 32,000.

PLATE 5

EXPLANATION OF FIGURES

15 During the coalescence of a typical mucous droplet (G) with a filamentous granule (F), the interface (arrow) between their respective contents is clear. Subsequently the filaments disintegrate. This breakdown of filaments must precede the extrusion of secretory material, since in no instance was a filamentous granule observed exiting from the cell into the acinar lumen. X 64,000.

16 Transfer of secretory material from the Golgi apparatus to the secretory granules occurs in two directions. On one side of the apparatus the cisternae contain a material similar in ultrastructure (arrows) to that found in ordinary mucous droplets. Cisternae along the opposite face of the apparatus, contain material (arrowheads) similar in appearance to the filaments. X 64,000.

17 This intercellular nerve terminal is characterized by a mixed population of vesicles, with some large dense-cored vesicles and, smaller electron-translucent vesicles. In addition, the terminals may contain neurotubules, bundles of microfilaments, and mitochondria. The membrane of this terminal is indicated by an arrow; the plasmalemma of the adjacent acinar secretory cell is identified by an arrowhead. X 55,000.

18 A portion of a myoepithelial cell showing the following organelles: N, nucleus; M, mitochondria; R, ribosomes; T, bundles of myofilaments. Both surfaces of myoepithelial cell, one facing the basal lamina, the other juxtaposed to a secretory cell (C), show considerable pinocytosis. X 19,000.

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